with no evidence of disease. Flow cytometry shows no aberrant populations. Cytogenetic testing is pending at this report. HTLV 1 viral load studies show consistent decrease in viral load. The patient is enjoying an active life with no significant post transplant adverse effects.

GVH/GVL

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HOST-REACTIVE MEMORY T LYMPHOCYTES ALONE DO NOT INDUCE MORE SEVERE GRAFT-VERSUS-HOST DISEASE

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Memory T lymphocytes are thought to mediate more rapid and profound memory immune response. In this study, we tested whether it is true in graft-versus-host disease (GVHD). C57BL/6 mice ($H2^b$) were injected with irradiated BALB/c ($H2^d$) spleen cells intraperitoneally at least 8 weeks before they were used as immunized donors. Splenic T cells (>90% pure) were harvested from these immunized animals and separated into $CD62L^+$ (>90% pure) and CD62L- (>99% pure) populations by magnetic beads. $CD62L^+$ T cells include both naive and memory T cells, while $CD62L^-$ T cells contain memory T cells but no naive T cells. We next compared the alloreactivity and the ability to induce GVHD (C57BL/6 to BALB/c) of $CD62L^-$ T cells with those of unseparated and $CD62L^+$ T cells. Despite $CD62L^-$ (memory) T cells contain similar numbers of interferon-γ-secreting cells upon response to host antigens as detected by enzyme-linked immunospot assay, $CD62L^-$ T cells had dramatically decreased ability to induce GVHD in BALB/c mice compared to unseparated and $CD62L^+$ T cell controls (Table). However, $CD62L^-$ T cell recipients did develop GVHD as documented by weight loss (Table) and histologic changes. Similar results were observed when the mice immunized with BCL1 cells (a BALB/c-origin B cell lymphoma/leukemia cell line) were used as donors. We conclude that host-reactive memory T cells alone have decreased ability to induce GVHD compared with a mixture of naive and memory T cells. Memory T cells obtained from tumor cell-immunized donors may be a unique source of T cells for tumor immunotherapy because they do not induce severe GVHD.

Groups	n	% Survival	% Weight loss
		day +100	day +100
TCD BM only	12	100%	9%
Unseparated T cells	25	4%	-
$CD62L^+$ T cells cells	4	0%	-
$CD62L^-$ T cells	9	89%	21%

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A CRITICAL ROLE FOR $CD4^+CD25^+$ SUPPRESSOR CELLS IN CONTROLLING GVHD AFTER DLI IN MURINE BONE MARROW CHIMERAS

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A murine model of allogeneic bone marrow (BM) transplantation (C57BL/6[B6]-into-AKR/J) was used to further define the role of $CD4^+CD25^+$ T cells in controling GVHD after DLI and their potential therapeutic application. B6-into-AKR BM chimeras were depleted of $CD25^+$ cells *in vivo* with anti-CD25 mAb or transplanted with BM from $CD25^{KO}$ donors so that the BM recipients would be devoid of donor-derived $CD25^+$ cells. Depletion of $CD25^+$ cells *in vivo* (>70% depletion) significantly increased the severity of DLI-induced GVHD as indicated by body weight loss and increased mortality as compared to nondepleted control mice. GVHD was even more intense in mice transplanted with $CD25^{KO}$ BM. Enhanced GVHD after CD25 depletion was seen after DLI of either 30 million or 60 million donor splenocytes. To test the ability of $CD25^+$ cells to persist and expand *in vivo*, nylon-wool enriched, immunomagnetically selected fresh $CD25^+$ cells were adoptively transferred into syngeneic nude mice. These cells progressively expanded (CD4 and CD8 subsets) over 5 months in syngeneic nude mice. Approximately 30% of the cells at 2-wk and less than 10% at 20-wk were still $CD25^+$. Various conditions for activation and expansion of $CD25^+$ suppressor cells were examined. Freshly-isolated B6 $CD25^+$ cells were cultured with rIL-2 in the presence of (1) immoblized anti-CD3 mAb plus soluble anti-CD28 mAb, (2) magnetic beads coated with anti-CD3 and anti-CD28 mAbs, and (3) CD32/4-1BB ligand double-transfected K562 tumor cells (K562DT) "loaded" with anti-CD3 and anti-CD28 mAbs. The K562DT cells induced superior short-term expansion of $CD4^+CD25^+$cells (>10 fold after 7-10 days of culture). Immoblized anti-CD3 plus soluble anti-CD28 preferentially expanded the CD8 subset from <3% in fresh $CD25^+$cells to >75% of the total cells after 1 week of culture. *Ex vivo* expanded $CD25^+$ cells were more suppressive than fresh cells added to primary mixed lymphocyte cultures in which 50% of stimulation inhibition was achieved at a 1:8 $CD25^+/CD25^-$ ratio for *ex vivo* expanded $CD25^+$ as compared to a 1:1 ratio required for fresh cells. Studies looking at the ability of *ex vivo* expanded $CD25^+$ cells to persist/expand *in vivo*, and the GVHD-protective role of freshly-isolated versus *ex vivo* expanded $CD25^+$ cells coadministered with DLI, are in progress.

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TCR TRANSGENIC HEMATOPOETIC STEM CELLS ENGRAFT INTO NON-TRANSGENIC RECIPIENTS AND EVOLVE ANTIGEN-SPECIFIC T-CELLS

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We hypothesized that genetically modified hematopoetic stem cells (HSC) transduced with a specific tumor antigen specific TCR may, when given as a fraction of a donor hematopoetic stem cell graft, lead to engraftment of tumor antigen specific T-cells and facilitate directed Graft vs Malignancy (GVL). We are developing a model system for exploring the feasibility of this approach. An important component of evaluating such a strategy is determining the ability for donor TCR-transgenic HSC to engraft into non-transgenic recipients, and to determine the kinetics of antigen specific T-cell engraftment. P14 mice are transgenic for the class-I restricted TCR specific to LCMV-Glycoprotein-33. Bone marrow was obtained from P14 CD45.2 TCR-Transgenic mice and transplanted into lethally irradiated non-transgenic, congenic CD45.1 recipients. Of the 500, 000 marrow cells in the graft, 0%, 10%, 25%, 50% or 100% was from a P14 donor, the remainder from CD45.1 congenic marrow. The number of LCMV-GP33 specific T-cells in the peripheral blood was measured weekly with MHC-H-2 D(b)GP33 tetramer. Control P14 mice contained an average of 25% CD8+ cells in the peripheral blood, of which 82% were GP33 tetramer positive. We found that TCR-transgenic P14 bone marrow would engraft, and result in measurable donor derived LCMV-GP specific CD8+ T-cells. The fraction of tetramer positive T-cells in peripheral blood increased in correlation to the percentage of P14 bone marrow transplanted. Four weeks post-transplant CD45.2, tetramer+ T-cells made up a mean 3.5% of peripheral blood in mice which had received 100% P14 donor marrow, 1.3% from 50% P14 donor, and .63% from a 25% P14 marrow donor. By week five, tetramer + cells had increased to 9.4% of blood cells in mice receiving 100% P14 donor marrow, 4.7% from 50% P14 marrow and .74% from 25% donor. Engraftment of tetramer+ T-cells increased in weeks 5 and 6 and then remained stable. 7 weeks post transplant in mice receiving 50% P14 marrow, 8.8% of peripheral cells were CD8+, of which 43.7% were tetramer+. 25% P14 transgenic donor recipients evolved 5.5% CD8+ T-cells of which 7.8% were tetramer positive. These results suggest that bone marrow containing TCR-

transgenic HSC can engraft and evolve significant numbers of antigen specific T-cells, which may in the future be utilized for targeted antigen specific GVL.

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RESIDUAL HOST CHIMERISM AND PATTERN OF ANTIGEN EXPRESSION INFLUENCE THE FATE OF ADOPTIVELY TRANSFERRED ALLOGENIC T CELLS

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Donor lymphocyte infusions (DLI) and allogeneic T cells specific for defined minor histocompatibility antigen (mHAg) are increasingly used in the treatment of relapsed malignancies after allogeneic BMT. However, the fate and function of adoptively transferred T cells are poorly understood. To evaluate the role of host chimerism, complete and mixed chimeras were constructed by transplanting DBA/2 mice (H-2^d) with bone marrow (BM) from MHC-mismatched C56BL/6 (H-2^b) donors after myeloablative and non-myeloablative conditioning, respectively. Three weeks later, both sets of chimeras received CFSE-labeled B6.SJL T cells, which uniquely express the CD45.1 allele. On day 6 and 10 after DLI administration, 86% and 93% of CD45.1$^+$ T cells in mixed chimeras, respectively, and 41% and 63% of CD45.1$^+$ T cells in full donor chimeras, respectively, underwent more than 7 divisions. Serial monitoring of CD45.1$^+$ T cells in full donor chimeras revealed that 28% of these cells remain undivided for more than 20 days. To define the effect of targeted antigen tissue expression pattern on the fate of allogeneic antigen-specific T cells, we used transgenic mice with wide (HA104) or pancreas-restricted (Ins-HA) expression of hemaglutinin (HA). TCR transgenic Clone 4 (CL4) mice, which contain H-2K^d restricted CD8$^+$ T cells specific for HA peptide were used as a source of antigen-specific T cells. HA104 or Ins-HA mice on BALB/c background underwent BMT from MHC-compatible B10.D2 donors, followed by DLI from TCR transgenic B10.D2-CL4 mice three weeks later. Differential expression of the Thy 1.1$^+$ antigen on transgenic T cells was used for in vivo monitoring of their fate. Adoptively transferred TCR-specific transgenic CD8$^+$ T-cells engrafted, expanded, and then sharply declined and became undetectable in the mice with wide distribution of targeted HA antigen. In contrast, transgenic T cells expanded but remained detectable for at least 30 days in the animals with pancreas-restricted HA expression without causing diabetes. However, co-administration of vaccinia virus expressing HA together with transgenic T cells resulted in diabetes. In conclusion, residual host chimerism, targeted antigens pattern of expression and antigen-specific vaccinations have a potential to influence the fate of adoptively transferred allogeneic T-cells.

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DONOR PRETREATMENT WITH G-CSF PREVENTS CHRONIC GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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The incidence of extensive chronic GVHD (cGVHD) after allogeneic stem cell transplantation with G-CSF mobilised peripheral blood stem cell (PBSC) products is increased compared to that after bone marrow transplantation and necessitates prolonged, expensive and poorly effective immunosuppressive therapy. G-CSF is known to alter T cell function and PBSC products contain 10-20 times the number of T cells relative to bone marrow grafts, both of which may increase cGVHD. We have studied the effects of G-CSF and T cell dose on cGVHD in two well-described models manifested by either immune complex glomerulonephritis (DBA/2→B6D2F1) or sclerodermatous hepatic and cutaneous fibrosis (B10.D2→Balb/c). The former model induces GVHD to major and minor HA and the latter to minor HA only. Donor mice were treated with G-CSF (2ug/day) or diluent for 6 days and recipient mice were transplanted with unseparated donor splenocytes that contained equal numbers of T cells. In the immune complex model, 80% of SCT recipients that received grafts from control treated donors develop nephrotic syndrome compared to 25% in the recipients of G-CSF treated grafts (P<0.01) and mortality was decreased from 45% to 15% (P<0.05). Donor T cell engraftment and B cell activation (class II expression) were equivalent in both groups. The reduction in cGVHD following donor G-CSF pretreatment occurred in the setting of donor Th2 differentiation manifested by reduced interferon-γ and increased IL-4 production with a concomitant 100-fold increase in the ratio of IgG1:IgG2α auto-antibody titres (P<0.05). In the scleroderma model, the effect of a 5-fold escalated donor G-CSF T cell dose (G-CSF-high T) was compared to the low T cell dose (G-CSF-low T and control-low T), to mirror the effect of the high T cell doses transferred during clinical peripheral blood stem cell transplantation. Hepatic and cutaneous semi-quantitative histopathology demonstrate that sclerodermatous cGVHD, characterised by epidermal thickening, fibrosis and procollagen deposition, is associated with high T cell doses but not G-CSF and Th2 differentiation per se (see table; *P<0.05, **P<0.01 v control). Thus, the limitation of T cell doses transferred during PBSCT should reduce chronic GVHD compared to BMT, whilst maintaining the beneficial effects on graft quality.

	Control allogeneic (low T)	G-CSF allogeneic (low T)	G-CSF allogeneic (high T)	Syngeneic
Hepatic GVHD	9.8±0.8	5.2±1.8*	7.8±1.1	1.2±0.3
Skin clinical score (0-4)	1.07±0.15	0.68±0.16	2.09±0.27**	0.0±0.0
Inflammatory score (0-24)	2.75±0.95	3.64±1.14	12.18±1.17**	0.22±0.01
Dermal collagen thickness (mm)	0.22±0.01	0.23±0.02	0.35±0.03**	0.23±0.01
Scleroderma % positive (n)	6% (16)	14% (14)	90% (10)**	0% (9)

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DELAYED INFUSION OF DONOR CD4$^+$25$^+$ T CELLS CONTROLS GRAFT-VERSUS-HOST DISEASE WHILE ALLOWING AN EFFECTIVE GRAFT-VERSUS-LEUKEMIA RESPONSE

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Following allogeneic hematopoietic cell transplantation (HCT), the high inverse correlation between graft-versus-host disease (GVHD) and leukemic relapse requires that calculated measures be taken to reduce GVHD pathology while retaining the graft-versus-leukemia effect (GVL). We sought to determine whether donor CD4$^+$CD25$^+$ regulatory T cells could control ongoing GVHD, effectively providing a window of time whereby the GVH response is permitted to begin in favor of the elimination of residual leukemia cells, before being brought under regulation to prevent the full development of lethal GVHD. Prevention of lethal GVHD by infusion of donor CD4$^+$CD25$^+$ T cells early post-HCT (day 2) could be achieved across an MHC barrier in the haploidentical C3H into (B6xC3H)F1 model. However, in vitro pre-sensitization of donor CD4$^+$CD25$^+$ T cells to recipient type alloantigen with high dose IL-2 was required for successful regulation. In contrast, in the minor histocompatability antigen (miHA)-disparate, CD8-mediated B10.BR into CBA GVHD model, lethal disease could be completely prevented by a single injection of freshly isolated donor CD4$^+$CD25$^+$ T cells, given as late as 10 days post-HCT. Of importance, this later regulatory effect required a CD4$^+$CD25$^+$ to effector CD8 ratio of only 1:3, indicating a strong potential for the delayed infusion of CD4$^+$CD25$^+$ T cells to control GVHD across miHA barriers. Furthermore, this regulation did not prevent complete and durable donor engraftment of the hematopoetic compartment. Of most significance, the day 10 infusion of donor CD4$^+$CD25$^+$ T cells into CBA HCT recipients that had been challenged with the